Three points concerning structure–activity relationship of these compounds can be made from these data: (1) the DMAE (**2**) series of compounds is consistently more potent than the DEO (**8**) series; (2) within a series, the bisquaternary compounds are consistently more potent than their monoquaternary counterparts; and (3) compounds possessing two phenyl rings are consistently more potent than those containing either one or three phenyl rings.

The DEO analogs appear to be less potent than the DMAE analogs due to the presence of ethoxyethyl rather than diethylacetal moieties. DEO itself also has a much shorter duration of action than DMAE, apparently the result of decreased affinity for its receptor.[7]

Just as hexamethonium is more potent than tetraethylammonium as a ganglionic blocker due to two-point rather than one-point binding,[13] probably these bisquaternary compounds are more potent than their monoquaternary counterparts for the same reason. The two- vs. one-point binding concept may be true even though the site of action within this atria preparation appears to be the adrenergic terminal rather than the ganglia.[14]

The greater potency of compounds with two phenyl rings over those possessing one or three rings may reflect the fact that this rigidly held intercationic distance of 14 Å is an ideal distance for binding. The 14-Å distance is a significant feature of neuromuscular blocking activity,[13] but its relevance in this study is not yet known. Because **5** and **11** are also fairly potent without the benefit of a second cationic head, the importance of the second phenyl ring itself must not be overlooked. This ring could be involved in binding or in blocking nicotine's approach to its receptor.

If this second ring is important, then one must account for the very low potencies of **6** and **12**. The third phenyl ring of **6** and **12** may be repulsed from the receptor surface by steric or electronic factors. However, **3** and **9** are again active because the second cationic head overcomes this repulsion by binding to the receptor surface. This would, of course, necessitate the presence of binding sites 18 Å apart in addition to those 14 Å apart. Further studies are needed to confirm or deny the presence of these binding sites.

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Methyl 5(6)-Phenylsulfinyl-2-benzimidazolecarbamate, a New, Potent Anthelmintic[1]

Eugene A. Averkin, Colin C. Beard,* Charles A. Dvorak, John A. Edwards, John H. Fried, John G. Kilian, Richard A. Schiltz,

Syntex Research, Palo Alto, California 94304

Theodore P. Kistner,

Corvallis, Oregon 97330

J. H. Drudge, Eugene T. Lyons,

Lexington, Kentucky 40500

Marvin L. Sharp,

Vernon, Texas 76384

and Robert M. Corwin

Columbia, Missouri 65201.

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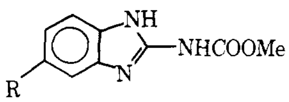
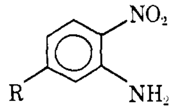
The preparation and anthelmintic properties of methyl 5(6)-phenylsulfinyl-2-benzimidazolecarbamate are described. It is effective at a dose of 10 mg/kg po against gastrointestinal nematodes in horses and at 5 mg/kg po or less against gastrointestinal nematodes and lungworms in cattle, sheep, and swine.

Benzimidazolecarbamates with anthelmintic activity have been reported by several groups of investigators. Alkyl[2] and benzoyl[3] substituents, for example, enhance this activity. During the course of our investigations we have found, as have others,[4,5] that benzimidazole-5(6) ethers and thioethers possess high activity against intestinal nematodes in laboratory animals. In addition, we have found that the 5(6)-phenylsulfinyl substituent confers particularly enhanced activity. Thus, methyl 5(6)-phenylsulfinyl-2-benzimidazolecarbamate (**1a**) is highly effective against a variety of parasites in laboratory and domestic animals.

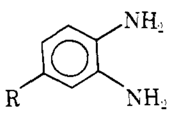


1a, R = PhS→O
b, R = PhS-
c, R = PhS→O_2



2a, R = PhS→O
b, R = PhS-
c, R = PhS→O_2

Chemistry. 2-Amino-4-phenylsulfenylnitrobenzene (**2b**) was obtained from 2-amino-4-chloronitrobenzene and thiophenol with potassium carbonate. The sulfoxide **2a** was prepared from the sulfide **2b** with 1 equiv of peracetic acid and gave the diamine **3a** upon hydrogenation in the presence of palladized charcoal. The benzimidazolecarbamate **1a** was obtained by reaction of the diamine **3a** with 1,3-bis(methoxycarbonyl-*S*-methyl)isothiourea.



3a, R = PhS→O
b, R = PhS-
c, R = PhS→O_2

Reduction of the aminonitro compound **2b** by iron–ferrous couple gave the diamine **3b**, which could also be obtained (slowly) by catalytic hydrogenation of **2b**. Displacement of the chlorine from 2-amino-4-chloronitrobenzene with sodium benzenesulfinate gave the sulfone **2c**, which was reduced catalytically to the diamine **3c**. Reaction of the diamines **3b** and **3c** with 1,3-bis(methoxycarbonyl-*S*-methyl)isothiourea gave the benzimidazole sulfide and sulfone, respectively. The sulfone **1c** could also be obtained by oxidation of the sulfide or sulfoxide with peracetic acid at the aminonitro- or benzimidazole stage. Similarly, oxidation of the benzimidazole sulfide **1b** with 1 equiv of peracid gave the sulfoxide **1a**.

Biological Data. In preliminary screening tests with mice, **1a**, administered in the feed for a period of 18 days,[6] showed effective removal of *Syphacia obvelata*, *Aspicularis tetraptera*, *Hymenolepis nana*, and *Nematospiroides dubius* at 31, 16, 125, and 62 ppm, respectively.

Compound **1a** was administered to sheep in single oral doses ranging from 0.3 to 15.0 mg/kg in "controlled tests". A dose of 5 mg/kg provides efficacy >95% against parasites of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Bunostomum*, *Capillaria*, *Cooperia*, *Nematodirus*, *Moniezia*, *Chabertia*, *Oesophagostomum*, and *Dictyocaulus*. A similar spectrum of activity was observed in cattle treated with 1.25–2.5 mg (**1a**)/kg. In horses, a dose as low as 1.1 mg/kg was effective in removal of *Strongylus vulgaris*, *Strongylus edentatus*, and mature *Oxyuris equi*. The same dose appears to be >95% effective against small *strongyles*. Consistent removal of *Parascaris equorum* is obtained at 10 mg/kg. Compound **1a** has no effect on bots. In swine, a dose of 3 mg/kg, administered in feed, was effective in the removal of *Ascaris suum*, *Oesophagostomum dentatum*, and *Metastrongylus sp.* No signs of toxicity were observed in any of the target species at doses at least five times the effective dose.

Preliminary acute toxicity studies indicated that the LD_{50} is over 1600 mg/kg for beagle dogs and over 6400 mg/kg in rats and mice. In each case, the dose was the maximum tested.

Table I[a]

Compd	ppm in diet	Av % reduction in no. of worms (<25 recorded as 0) *N. dubius*	*H. nana*	*S. obvelata*	*A. tetraptera*
1a	125	100	100	100	100
	62.5	93	0	100	100
1b	250	96	34	100	100
	125	62	0	100	100
1c	500	77	0	0	100
Thiabendazole	500	91	0	100	70
	250	31	0	100	0
	125	0	0	0	0

[a]These results were obtained by the method of Brody and Elward.[6] There were four mice per treated group, although for compound **1a** the results are from several groups of four mice.

The high activity of compound **1a** in the primary (mouse) assay (Table I) is also realized in sheep, cattle, horses, and swine, the "target" domestic animals. Compared to the corresponding sulfide **1b**, compound **1a** has greater effect in the mouse against *N. dubius* and *H. nana* (Table I), shows comparable activity in sheep, and also is 2–3 times as potent in cattle.[5] The corresponding sulfone **1c** is considerably less active in the mouse assay. Data for thiabendazole are also given in Table I for comparison (see also ref 6 for other standard drugs).

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet spectra were determined in methanol with a Cary 14 instrument. Infrared spectra were obtained in KBr with a Perkin-Elmer 237B spectrometer. NMR spectra were obtained with Varian A-60 and HA-100 instruments, and mass spectra were determined with a Varian-MAT CH4 spectrometer. Elemental analyses were performed by the analytical department of Syntex Research, Institute of Organic Chemistry, and are within ±0.4% of calculated values.

2-Amino-4-phenylsulfenylnitrobenzene (2b). A mixture of 5-chloro-2-nitroaniline (3.5 g, 0.02 mol), thiophenol (2.2 g, 0.02 mol), and potassium carbonate (4.14 g, 0.03 mol) in 15 ml of dimethylformamide was heated at 100° under nitrogen for 1 hr. The cooled mixture was poured into 300 ml of water, and the precipitate was collected and washed with water: yield 4.78 g (91%) of **2b**. Recrystallization from 2-propanol gave pure **2b**, mp 118–119°.

2-Amino-4-phenylsulfinylnitrobenzene (2a). A solution of 2-amino-4-phenylsulfenylnitrobenzene (**2b**) (3.0 g, 0.012 mol) in chloroform (30 ml) was treated at −10 to −5° with peracetic acid (2.1 g, 0.012 mol, ~40% commercial material, FMC Corp.). The solution was allowed to warm to 20–25°, then washed with sodium bicarbonate solution and water, and dried. The solvent was evaporated and the residue recrystallized from methanol: yield 2.88 g (92%) of **2a**; mp 137–138°.

4-Phenylsulfinyl-*o*-phenylenediamine (3a). A solution of 2-amino-4-phenylsulfinylnitrobenzene (**2a**) (10.1 g, 0.039 mol) in methanol (100 ml) was hydrogenated under ambient conditions in the presence of 5% palladized charcoal (2.5 g) (~2 hr). The catalyst was filtered off and the methanol solution concentrated. Toluene was added and the solution concentrated further. Cooling gave pure diamine (8.6 g, 95%) **3a**, isolated by filtration: mp 141–143°. Anal. ($C_{12}H_{12}N_2OS$) C, H, N, S.

Methyl 5(6)-Phenylsulfinyl-2-benzimidazolecarbamate (1a). A solution of 4-phenylsulfinyl-*o*-phenylenediamine (**3a**) (1.16 g, 0.005 mol), 1,3-bis(methoxycarbonyl-*S*-methyl)isothiourea (1.13 g, 0.005 mol), and acetic acid (0.36 ml, 0.006 mol) in ethanol (20 ml) and water (20 ml) was refluxed for 3 hr. The mixture was cooled and essentially pure product (**1a**) filtered off (1.49 g, 94.5%). Recrystallization from chloroform–methanol gave purified material: mp ~253° dec.[7] Anal. ($C_{15}H_{13}N_3O_3S$) C, H, N.

4-Phenylsulfenyl-*o*-phenylenediamine (3b). A solution of 2-amino-4-phenylsulfenylnitrobenzene (**2b**) (1.8 g, 0.007 mol) in methanol (160 ml) and water (40 ml) containing ferrous sulfate heptahydrate (1.0 g, 0.004 mol) was refluxed and treated with iron powder (2.0 g). Two further portions of iron were added at 2 and 4 hr. The reaction was finished in ~5–6 hr, when insoluble material was filtered off. The filtrate was evaporated and the residue taken up in benzene. The benzene solution was filtered and evaporated, leaving the diamine **3b** as a gum (1.4 g, 90%) which later crystallized: mp ~53–55°.

Methyl 5(6)-Phenylsulfenyl-2-benzimidazolecarbamate (1b). 4-Phenylsulfenyl-*o*-phenylenediamine (**3b**) (0.7 g, 0.0032 mol) was treated with 1,3-bis(methoxycarbonyl-*S*-methyl)isothiourea (110%), as described for the preparation of the sulfoxide **1a**. Recrystallization from methanol–chloroform gave the benzimidazole **1b**: mp 243° dec. Anal. ($C_{15}H_{13}N_3O_2S$) C, H, N.

2-Amino-4-phenylsulfonylnitrobenzene (2c). A mixture of 5-chloro-2-nitroaniline (2.0 g, 0.011 mol) and sodium benzenesulfinate (5.0 g, 0.03 mol) in dimethylformamide was heated at ~150–160° for 3.5 hr. After cooling, the mixture was diluted with water and the product filtered off: mp 180–182.5° (3.0 g, 98%).

4-Phenylsulfonyl-*o*-phenylenediamine (3c). A solution of the nitroamine **2c** (1.9 g, 0.007 mol) in methanol was hydrogenated at 3 atm of pressure in the presence of Raney nickel catalyst for 2 hr at 20°. The catalyst was filtered off and the filtrate evaporated. The residue was recrystallized from benzene, yielding pure diamine **3c** (1.53 g, 90%): mp 112.5–113.5°.

Methyl 5(6)-Phenylsulfonyl-2-benzimidazolecarbamate (1c). 4-Phenylsulfonyl-*o*-phenylenediamine (**3c**) (0.75 g, 0.03 mol) was treated with 1,3-bis(methoxycarbonyl-*S*-methyl)isothiourea (0.68 g, 0.033 mol), as described above for the preparation of **1a**. Recrystallization from methanol–chloroform gave the pure benzimidazole **1c**: mp >320° (0.86 g, 87%). Anal. ($C_{15}H_{13}N_3SO_4$) C, H, N. This compound may also be prepared by the oxidation of the sulfoxide **1a** or the sulfide **1b** with peracetic acid (1 and 2 equiv, respectively) in chloroform–acetic acid. Solvent is removed by evaporation under vacuum and the residue treated with sodium bicarbonate solution. The product is filtered off, washed with water, and recrystallized from methanol–chloroform.

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(7) Inserted in an oil bath at ~250°, the melting point is ~253° dec, followed by resolidification, then remelting at ~275–278°; the initial decomposition at ~253° is not apparent if the sample is inserted in the oil bath below about 245°.

Synthesis and Antibacterial Properties of Methylsulfinyl and Methylsulfonyl Analogs of Some Nitrofurans[1]

Bertil Ekström, Maria Ovesson, and Brian G. Pring*

Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden. *Received June 27, 1975*

The sulfoxides 5-methylsulfinyl-2-furaldehyde semicarbazone (**2**) and 1-[(5-methylsulfinyl-2-furfurylidene)amino]hydantoin (**3**) as well as the sulfones 1-[(5-methylsulfonyl-2-furfurylidene)amino]hydantoin (**1**) and 1-(5-methylsulfonyl-2-furyl)-2-(6-amino-3-pyridazyl)ethylene hydrochloride (**4**) have been prepared and tested for antibacterial activity against a number of gram-negative and gram-positive organisms. The compounds are much less active than the corresponding 5-nitrofuran derivatives, possibly because their reduction potentials are too negative for them to interfere with reductive enzyme systems within the bacteria.

During the past three decades, numerous derivatives of 5-nitrofuran substituted at the 2 position have been prepared, many of them with high antibacterial activity.[2] More recently, analogs of the more active nitrofurans have been prepared in which the nitro group has been replaced by other electronegative groups such as trifluoromethyl,[3] aryl-, aralkyl- and alkylsulfonyl,[4–6] cyano[7,8] and sulfo, sulfamoyl, carboxyl, methoxycarbonyl, and carbamoyl.[8] None of these compounds were reported to possess significant antibacterial activity, although bactericidal properties have been claimed for the methylsulfonyl analog of nitrofurantoin (**1**) and certain of its derivatives.[9]

Nitrofurans are reduced by a number of enzyme systems present in bacteria[10,11] and the ease of reduction of the nitro group may be correlated to the antibacterial activity.[12] We considered that analogs of nitrofurans containing a reducible group with similar electronic properties to nitro might possess antibacterial activity and have therefore prepared two compounds containing a sulfoxide group, namely the methylsulfinyl analogs of nitrofurazone and nitrofurantoin (compounds **2** and **3**), respectively. The methylsulfonyl analogs of nitrofurantoin and nifurprazine, a nitrofuran of the vinylogous imine type with enhanced bacterial activity,[13] have also been prepared (compounds **1** and **4**, respectively).

Synthesis. The nitrofurazone and nitrofurantoin analogs (Table I) were prepared by condensing the appropriate furaldehyde with semicarbazide or 1-aminohydantoin.[14] In order to prepare 5-methylsulfinyl-2-furaldehyde (**5**), 5-bromo-2-furaldehyde was converted to the methylmercaptan[4] which was oxidized to the sulfoxide with sodium periodate. The methylsulfonyl analog of nifurprazine (**4**, Table I) was prepared by condensing 5-methylsulfonyl-2-furaldehyde[5] with 3-acetamido-6-methylpyridazine[15] in the presence of $AcOH–Ac_2O$ followed by removal of the acetyl group by acidic hydrolysis. An attempt to prepare the methylsulfinyl analog of nifurprazine by this procedure was unsuccessful.

Antibacterial Activity. The compounds were tested for antibacterial activity in vitro using a standard agar dilution technique[16] against a number of gram-negative and gram-positive organisms including *Escherichia coli*, *Klebsiella*